

University of Groningen

Mutacin II, a Bactericidal Lantibiotic from *Streptococcus mutans*

Chikindas, Michael L.; Novák, Jan; Driessen, Arnold J.M.; Konings, Wilhelmus; Schilling, Kurt M.; Caufield, Page W.

Published in:
Antimicrobial Agents and Chemotherapy

DOI:
[10.1128/aac.39.12.2656](https://doi.org/10.1128/aac.39.12.2656)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1995

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Chikindas, M. L., Novák, J., Driessen, A. J. M., Konings, W., Schilling, K. M., & Caufield, P. W. (1995). Mutacin II, a Bactericidal Lantibiotic from *Streptococcus mutans*. *Antimicrobial Agents and Chemotherapy*, 39(12), 2656-2660. <https://doi.org/10.1128/aac.39.12.2656>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Mutacin II, a Bactericidal Lantibiotic from *Streptococcus mutans*

MICHAEL L. CHIKINDAS,^{1†} JAN NOVÁK,² ARNOLD J. M. DRIESSEN,^{3*} WIL N. KONINGS,³
KURT M. SCHILLING,^{1‡} AND PAGE W. CAUFIELD²

Unilever Dental Research, Beblington L63 3JW, United Kingdom¹; Department of Oral Biology, School of Dentistry,
University of Alabama at Birmingham, Birmingham, Alabama 35294²; and Department of Microbiology,
Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen,
9751 NN Haren, The Netherlands³

Received 7 August 1995/Returned for modification 31 August 1995/Accepted 10 October 1995

Mutacin II is a lantibiotic that is produced by group II *Streptococcus mutans*. It inhibits the growth of other streptococci as well as many other gram-positive microorganisms by a hitherto unknown mechanism. Mutacin II possesses bactericidal activity against susceptible cells. It transiently depolarizes the transmembrane electrical potential ($\Delta\psi$) and the transmembrane pH gradient (ΔpH) and partially inhibits amino acid transport. However, it rapidly depletes the intracellular ATP pool in glucose-energized cells and prevents the generation of ATP. It is concluded that mutacin II does not belong to the group of pore-forming lantibiotics (type A) or to the type B lantibiotics, which inhibit phospholipases or interfere with peptidoglycan biosynthesis. Mutacin II acts by inhibiting essential enzyme functions at the level of metabolic energy generation, an activity that has not yet been classified for lantibiotics.

Mutans streptococci as well as many other microorganisms which inhabit the oral cavity produce antimicrobial substances of a proteinaceous nature (13). These are referred to as bacteriocins (27) or bacteriocin-like inhibitory substances (32). The term “mutacin” has been proposed for antimicrobial peptides originating from *Streptococcus mutans* (8). These antimicrobial peptides can play significant roles in primary colonization and give a competitive advantage to producer strains occupying critical ecological niches in dental plaque. Therefore, bacteriocins have several potential biotechnological applications and have been explored for their ability to provide protection against bacteria causing dental diseases (10, 11).

Recently, mutacin II was isolated from *S. mutans* T8 and partially characterized (21). It is a small (3,245 Da) peptide, and on the basis of chemical modification techniques and ion spray mass spectroscopy, it contains one β -methyllanthionine and two lanthionines in addition to a dihydro amino acid (22). These data suggest that mutacin II belongs to the lantibiotic group of bacteriocins (12). Mutacin II is stable and biologically active over a wide range of pH values and temperatures (21).

In this study the mode of action of mutacin II has been analyzed. The results suggest that mutacin II acts in a bactericidal manner and that it interferes with the capacity of cells to generate metabolic energy. Its biological activity appears to be directed at the inhibition of enzyme functions.

MATERIALS AND METHODS

Bacterial strains and media. *S. mutans* NTCC 10449, *Streptococcus sanguis* Ny101 (21), and *Lactococcus lactis* subsp. *lactis* IL1403 (4) were used as mutacin II-susceptible strains. Cells were grown in brain heart infusion broth or agar

(Difco Laboratories, Detroit, Mich.) at 30°C (for *L. lactis*) or at 37°C (for the *Streptococcus* spp. and *Escherichia coli*) without aeration. Purified nisin was purchased from NBS Biologicals (North Mymms, Hatfield, Herts, United Kingdom) or obtained from Aplin & Barrett and stored at –20°C in acetic acid solution (pH 3.0) at 10 mg/ml.

Purification of mutacin II and determination of its biological activity. Mutacin II was purified as described by Novák et al. (21). Bacteriocin activity in prepared samples was determined by the agar spot tests (9). In short, 10 μ l of a solution containing bacteriocin was spotted on an agar plate in serial twofold dilutions in demineralized water containing 0.5% (vol/vol) Tween 20. After the liquid had been completely absorbed by the agar, a top agar layer seeded with 0.1% of an overnight culture of susceptible *S. sanguis* Ny101 was poured over the plates. The plates were incubated for 16 h at 37°C and examined for zones of growth inhibition. The highest dilution which produced a distinct zone of inhibition after this incubation period was defined as 1 arbitrary unit (AU). One arbitrary unit corresponded to about 0.035 μ g of purified mutacin II and 0.025 μ g of purified nisin.

Effect of mutacin II on growth of susceptible cells. Overnight cultures were diluted to approximately 6×10^6 cells per ml and tested for their susceptibility to different concentrations of mutacin II. Briefly, aliquots of 0.1-ml samples were taken at 30-min intervals to determine the viable count by plating onto brain heart infusion agar. In order to detect lytic activity, optical density (OD) was determined either at 595 nm (Bio-kinetics reader EL 312e; Bio-Tek Instruments) or at 630 nm (DIAS microplate reader; Dynatech Laboratories, Guernsey, United Kingdom), depending on the instrument used in the experiment.

Measurement of the transmembrane electrical potential. The transmembrane electrical potential ($\Delta\psi$) in cells was determined with an electrode specific for the lipophilic cation tetraphenylphosphonium (31) as described previously (3).

Measurement of intracellular pH. The intracellular pH was determined by the use of the fluorescent pH probe 2',7'-bis-(2-carboxyethyl)-5[and 6]-carboxyfluorescein (BCECF; Molecular Probes, Inc., Eugene, Oreg.), which was trapped in *L. lactis* IL1403 cells by an acid shock (17). Cells suspended in 50 mM potassium phosphate (pH 6.0) were energized with 0.5% (wt/vol) glucose at 30°C, and the BCECF fluorescence was monitored with a Perkin-Elmer LS50B spectrofluorimeter with computer-controlled data acquisition and storage using excitation and emission wavelengths of 502 and 525 nm, respectively. The excitation and emission slit widths were 5.0 and 15.0 nm, respectively. Data were corrected for ATP-driven efflux of the fluorescent probe (18), and the fluorescent signal was calibrated by measuring the fluorescence at different pH values in the presence of 1 μ M nigericin to equilibrate protons across the membrane (17). ΔpH was calculated from the difference between intracellular pH and medium pH.

Transport assays. The uptake of the amino acids [1-¹⁴C]- α -aminoisobutyric acid (AIB) (8.5 μ M; 59 mCi/mmol) and L-[U-¹⁴C]glutamate (1.75 μ M; 285 mCi/mmol) was analyzed as described previously (3).

Determination of intracellular ATP concentration. Cells were harvested at late exponential growth phase (OD at 600 nm [OD₆₀₀] of 1.2), washed once with 100 mM potassium phosphate (pH 6.5), and concentrated to an OD₆₀₀ of about 3.0 (23). The cell suspension was energized with 0.5% (wt/vol) glucose, and aliquots of the suspension were taken at specific time points. Samples were mixed with half a volume of 14% (wt/vol) perchloric acid–9 mM Na₂EDTA. The supernatant was neutralized with half a volume of 1 M KOH–1 M KHCO₃. The

* Corresponding author. Mailing address: Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands. Phone: 31 50 632164. Fax: 31 50 635154. Electronic mail address: A.J.M.DRIESSEN@BIOL.RUG.NL.

† Present address: Department of Bacteriology and Mycology, Janssen Research Foundation, Division of Janssen Pharmaceutica N.V., B-2340 Beerse, Belgium.

‡ Present address: Unilever Research US Inc., Edgewater NJ 07020.

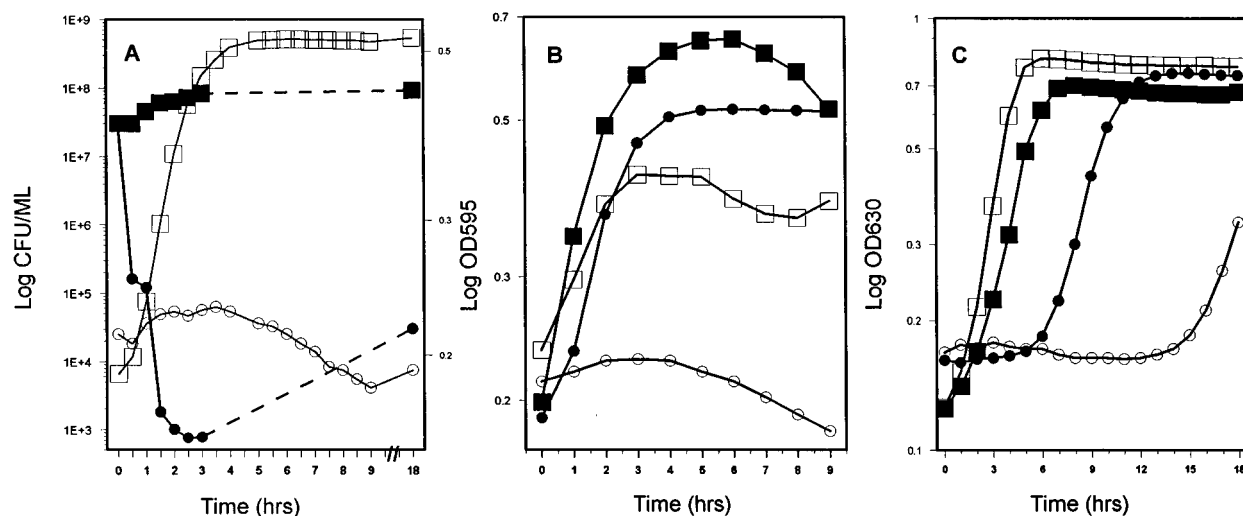


FIG. 1. Mutacin II activity against susceptible lactic acid bacteria. (A) Mutacin II possesses bactericidal activity against *S. sanguis* Ny101 cells. Symbols: \square , log OD₅₉₅ of control cells (no additions); \blacksquare , log CFU of control cells (no additions) per ml; \circ , log OD₅₉₅ of cells treated with 10 AU of mutacin II per ml; \bullet , per ml, log CFU of cells treated with 10 AU of mutacin II per ml. (B) Spontaneous occurrence of mutacin II-resistant *S. sanguis* Ny101 cells. Partially resistant Ny101 cells (Ny101R) were obtained by growing NY101 cells in the presence of 10 AU of mutacin per ml for 18 h. Ny101R (squares) or Ny101 (circles) cells were inoculated onto fresh medium with (\square and \circ) and without (\blacksquare and \bullet) 30 AU of mutacin II per ml, and the log OD₅₉₅ was monitored. (C) Synergistic antibacterial effect of mutacin II and the surfactant cocolactobionamide on *S. mutans* NTCC 10449. Symbols: \square , control cells (no additions); \blacksquare , 0.005% (wt/vol) cocolactobionamide; \bullet , 8.25 AU of mutacin II per ml; \circ , 0.005% (wt/vol) cocolactobionamide and 8.25 AU of mutacin II per ml. Growth was monitored by measuring the OD₆₃₀.

neutralized extract was diluted 40-fold into 40 mM Tris-SO₄, and just before the measurement 2 mM EDTA (pH 7.75) and 10 mM MgSO₄ were added. ATP was measured by the firefly luciferase assay as described previously (23).

RESULTS

Mutacin II is a bactericidal lantibiotic. To investigate the mode of action of the lantibiotic mutacin II against the susceptible cells, an overnight culture of *S. sanguis* Ny101 was diluted in fresh brain heart infusion broth with and without 10 AU of mutacin II per ml and the viable count and optical density changes were determined as a function of time. After 1 h of incubation in the presence of mutacin II, 96% of the cells lost their ability to form colonies (Fig. 1A). After 3 h of treatment only 0.003% of the cells survived, but further incubation (i.e., 18 h) resulted in increases in both OD and colony forming ability, suggesting the appearance of resistant cells. Cell lysis, as detected by a change in OD of the cells, was not observed during the time of experiment. Stationary-phase (18 h) Ny101 cells were at least twofold more susceptible to mutacin II than exponentially growing cells (6 h) (data not shown). These data suggest that mutacin II acts in a bactericidal manner.

After prolonged (18 h) growth in the presence of the bacteriocin, a subpopulation of Ny101 cells was able to grow. Rapid growth of these cells (Ny101R) was observed when they were incubated in fresh growth medium, both in the absence (Fig. 1B, solid boxes) and in the presence (open boxes) of 30 AU of mutacin II per ml. This apparent resistance was lost when the cells were grown for 24 h in the absence of the bacteriocin (data not shown).

The surfactant cocolactobionamide enhances the bactericidal activity of mutacin II. Some lantibiotics act at the level of the cytoplasmic membrane of susceptible cells (7), and their activity may be enhanced in the presence of surfactants that are thought to increase access to the phospholipids (5). Growth of susceptible *S. mutans* NTCC 10449 cells was monitored following treatment with a partially inhibiting concentration of mutacin II, the surfactant cocolactobionamide (1), and a combination of both. The data shown in Fig. 1C dem-

onstrate that the surfactant has a synergistic antibacterial effect when added in combination with mutacin II, whereas little inhibition of growth is observed with surfactant alone.

Mutacin II transiently and partially depolarizes the transmembrane electrical potential and pH gradient. To study whether mutacin II was capable of permeabilizing the cytoplasmic membrane of susceptible cells, its effect on the transmembrane electrical potential ($\Delta\psi$) was determined by monitoring the distribution of the lipophilic cation tetraphenylphosphonium in energized cells. The $\Delta\psi$ of susceptible *S. sanguis* Ny101 cells that were either treated (Fig. 2B) or not treated (data not shown) with nigericin was transiently depolarized by 30 AU of mutacin II per ml. However, the dissipation was not complete, and it was only temporary. Under the same set of conditions,

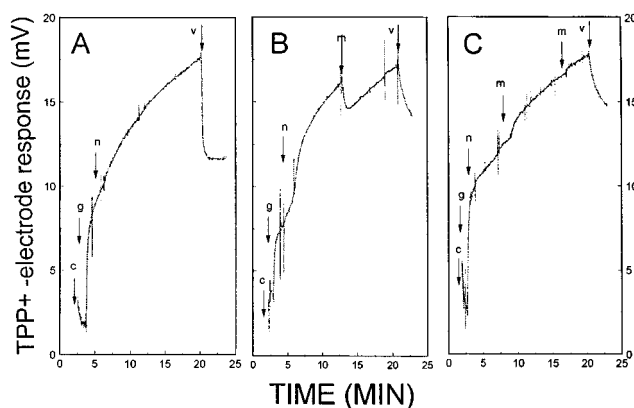


FIG. 2. Effect of mutacin II on the $\Delta\psi$ of glucose-energized cells of *S. sanguis* Ny101. (A) Control; (B) cells treated with 30 AU of mutacin II per ml; (C) cells pretreated with 30 AU of mutacin II per ml. Arrows indicate the following additions: c, cells; g, 0.4% (wt/vol) glucose; n, 1.0 μ M nigericin; v, 1.0 μ M valinomycin; and m, 30 AU of mutacin II per ml. The traces show the response of the tetraphenylphosphonium ion (TPP⁺)-selective electrode as a qualitative measure of the $\Delta\psi$.

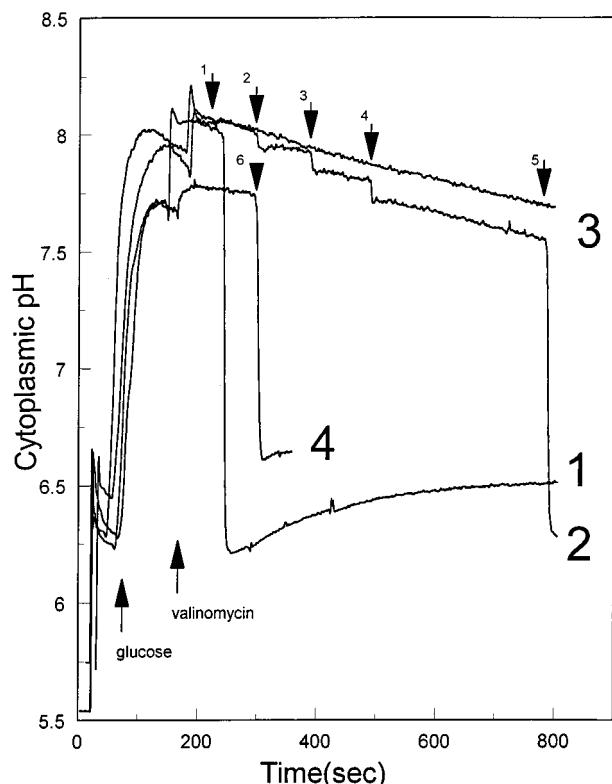


FIG. 3. Effect of mutacin II on the Δ pH of glucose-energized cells of *L. lactis* IL1403. Cells were loaded with the fluorescent pH indicator BCECF to record intracellular pH changes as described in Materials and Methods. Glucose (0.5% [wt/vol]) and valinomycin (1.0 μ M) were added at the respective arrows. At the numbered arrows the following sequential additions were made: trace 1, nigericin (1.0 μ M) at time point 1; trace 2, 50 AU of mutacin II per ml at time points 2, 3, and 4 and 1.0 μ M nigericin at time point 5; trace 3, no additions; and trace 4, 50 AU of nisin per ml at time point 6. The fluorescence level was converted into intracellular pH values for the cells after calibration and correction for BCECF efflux.

30 AU of nisin per ml completely collapsed the $\Delta\psi$ (data not shown) (7). The $\Delta\psi$ recovered shortly after the cells were treated with mutacin II. In contrast, the potassium ionophore valinomycin elicited a complete dissipation of the $\Delta\psi$ (Fig. 2A). When susceptible cells were preincubated with mutacin II before the energy source glucose was added, no significant effect of repeated additions of mutacin II on the $\Delta\psi$ was observed (Fig. 2C). In no instance was a complete depolarization of the $\Delta\psi$ by mutacin II observed. Similar results were obtained with susceptible *L. lactis* IL1403 cells, whereas the $\Delta\psi$ of resistant *S. sanguis* Ny101 cells (Ny101R) was not affected by mutacin II (data not shown). These results suggest that mutacin II only temporarily affects the ion permeability of the cytoplasmic membrane of susceptible cells and that it does not form stable pores.

To study the effect of mutacin II on the transmembrane pH gradient (Δ pH) of susceptible cells, *L. lactis* IL1403 cells were loaded with the fluorescent pH indicator BCECF to monitor the intracellular pH. When cells were energized with glucose, a rapid increase in the intracellular pH was observed (Fig. 3). Dissipation of the $\Delta\psi$ by valinomycin (1 μ M) resulted in a further increase in the intracellular pH. Addition of nigericin (1 μ M), a K^+/H^+ ionophore (Fig. 3, trace 1), or the lantibiotic nisin (30 AU/ml) (trace 4) rapidly reversed the intracellular pH to a level equal to that of the medium pH. Four repeated

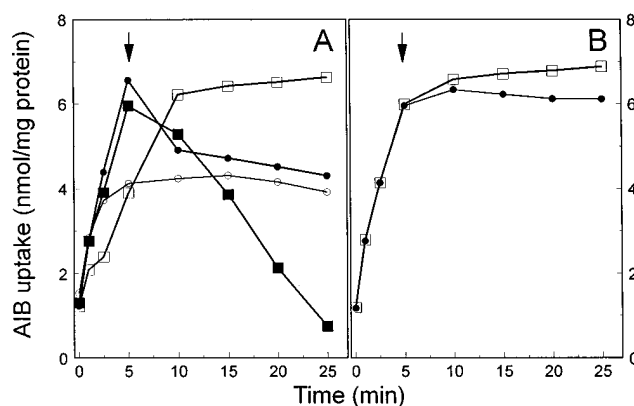


FIG. 4. Effect of mutacin II on AIB uptake by glucose-energized cells of *S. sanguis* Ny101 (A) and Ny101R (B). Symbols: \square , control cells (no additions); \blacksquare , 1 μ M valinomycin and 1 μ M nigericin added at the time indicated by the arrow; \bullet , 100 AU of mutacin II per ml added at the time indicated by the arrow; \circ , cells pretreated with 100 AU of mutacin II per ml for 1 min prior to the addition of AIB.

additions of 50 AU of mutacin II per ml each (trace 2) or one single addition of 200 AU of mutacin II per ml (data not shown) caused a small but reproducible dissipation of the pH gradient. These results indicate that mutacin II has only a minor effect on the proton permeability of the cytoplasmic membrane.

Mutacin II inhibits the uptake of amino acids. The influence of mutacin II on the proton motive force-dependent uptake of AIB (6, 15), a nonmetabolizable analog of alanine (33), by energized susceptible (Ny101) (Fig. 4A) and partially resistant (Ny101R) cells (Fig. 4B) was studied. AIB is rapidly accumulated by both Ny101 and Ny101R cells. Addition of valinomycin and nigericin (1 μ M each) (Fig. 4A, solid boxes; added at the arrow) caused a slow but complete release of the accumulated AIB. On the other hand, addition of mutacin II (100 AU/ml; solid circles) resulted in only a partial release of accumulated AIB to the level observed when the cells were preincubated with mutacin prior to the addition of AIB (open circles). Mutacin II hardly affected the uptake of AIB by Ny101R cells (Fig. 4B). The partial inhibition of AIB uptake in susceptible cells is consistent with the observation that mutacin II elicits a partial collapse of proton motive force.

Glutamate uptake by lactic acid bacteria is a phosphate-bond-linked (ATP-driven), unidirectional process that is not dependent on the proton motive force (24, 25). Cells of Ny101 efficiently retained the accumulated glutamate upon the addition of the ionophores valinomycin and nigericin (1 μ M each) (Fig. 5, diamonds; added at arrow A), but complete release was effected by the pore forming lantibiotic nisin (solid boxes). In contrast, mutacin II (100 AU/ml) (solid circles) again caused only a partial release of the accumulated glutamate to the level that was observed when cells were preincubated with mutacin II prior to the addition of glutamate (open circles). Prior addition of valinomycin and nigericin had little effect on the mutacin II-induced release of glutamate, indicating that the proton motive force is not required for functioning of mutacin II. Mutacin II did not elicit glutamate release from Ny101R cells (data not shown). These results demonstrate that mutacin II transiently permeabilizes the membrane and that this permeabilization is accompanied by a short burst in which solutes like glutamate are released.

Mutacin II depletes the intracellular ATP pool in glucose-energized cells. To investigate whether mutacin II affects the

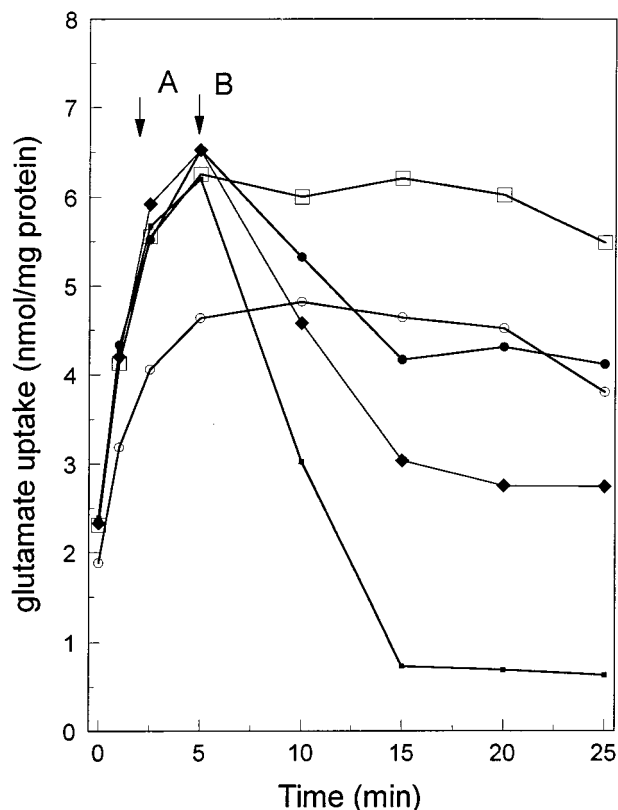


FIG. 5. Effect of mutacin II on L-glutamate uptake by glucose-energized cells of *S. sanguis* Ny101. Symbols: \square , control cells (no additions); \bullet , 100 AU of mutacin II per ml added at the time indicated by arrow B; \blacklozenge , 1 μ M valinomycin and 1 μ M nigericin added at the time indicated by arrow A and 100 AU of mutacin II per ml added at the time indicated by arrow B; \circ , cells pretreated with 100 AU of mutacin II per ml (1 min) prior to the addition of glutamate; \blacksquare , 100 AU of nisin per ml added at the time indicated by arrow B.

level of intracellular ATP, susceptible *L. lactis* IL1403 cells were energized with glucose and the impact of 100 AU of mutacin II per ml on the ATP level was measured. Addition of mutacin II to the cells resulted in a nearly complete depletion of the intracellular ATP pool (Fig. 6). In cells preincubated with mutacin II, virtually no ATP was generated upon the addition of glucose. These data demonstrate that mutacin II depletes the entire intracellular ATP pool and thereby dramatically affects the energetic status of susceptible cells.

DISCUSSION

Mutacin II is a bacteriocin produced by some strains of oral streptococci (32). In this study the effect of mutacin II on susceptible cells was analyzed. Mutacin II is bactericidal, but the mechanism of its activity is not yet clear. A transient and only partial dissipation of the $\Delta\psi$ and ΔpH occurred upon the addition of mutacin II. This effect was accompanied by a partial release of AIB and glutamate. Further additions of mutacin II did not result in a greater effect. In vivo labelling experiments using intracellular DNA with the specific membrane-impermeable fluorescent label propidium iodide demonstrated that mutacin II does not cause the disruption of the cells or a subset thereof (2). Therefore, it appears that the initial interaction of mutacin with the cells is responsible for the transient and incomplete permeabilization of the membrane for ions and small solutes. The principal biological activity of mutacin

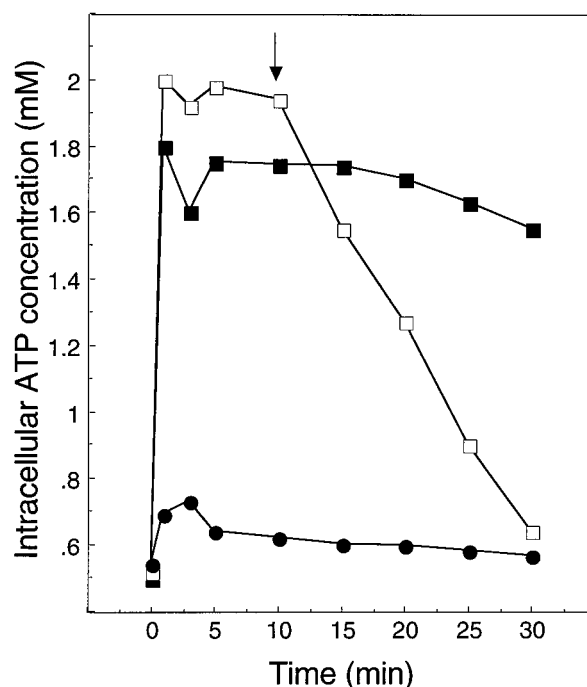


FIG. 6. Effect of mutacin II on the intracellular ATP level in glucose-energized cells of *L. lactis* IL1403. Symbols: \blacksquare , control cells (no additions); \square , 100 AU of mutacin II per ml added at the time point indicated by the arrow; \bullet , cells pretreated with 100 AU of mutacin II per ml for 1 min prior to the addition of 0.5% (wt/vol) glucose.

II appears not to rely on pore formation, in contrast to the activity of type A lantibiotics such as nisin (7, 28–30). A dramatic effect of mutacin II on the cellular ATP levels was observed. Addition of mutacin II caused a complete depletion of the intracellular ATP pool. Therefore, it seems likely that the partial depolarization of the proton motive force is the indirect result of the permeation of these molecules across the cytoplasmic membrane to gain access to the cytosol. There, it may interact with the primary target, for instance, with enzymes involved in the generation of metabolic energy at the substrate level. The mechanism by which mutacin II depletes the intracellular ATP pool is so far obscure. Mutacin may either inhibit the enzymes involved in ATP generation or activate ATPases to induce futile cycling and a depletion of available ATP. In this respect, barely any effect of mutacin II on the rate of glycolysis (as measured by the rate of acid production) of lactococcal cells (2) or on the uptake of the nonmetabolizable carbohydrate analog methyl- β -D-thiogalactopyranoside (19) was observed. On the other hand, mutacin II also does not appear to belong to the group of type B lantibiotics, which contains, e.g., cinnamycin, duramycin, mersacidin, and actagardine (28), globular polypeptides that act as enzyme inhibitors. The primary target of these type B lantibiotics is peptidoglycan synthesis or inhibition of phospholipases. The primary target of mutacin II seems not to be related to either of these phenomena; rather, mutacin II seems to be directed at the inhibition of metabolic energy generation.

When cells are grown in the presence of mutacin II, a spontaneous development of cells that appear to be resistant to the lantibiotic occurs. Intriguingly, this phenomenon is not a stable attribute, since it requires that the cells remain exposed to the lantibiotic. The mechanism of this apparent resistance is not known, but the spontaneous occurrence suggests that there is

heterotypy among the bacteria as far as their susceptibility to mutacin II is concerned. This may limit the use of mutacin II in dental applications. A phenotypically similar resistance has recently been described for pediocin AcH (20). In the natural environment, however, the bacterium-killing effect of mutacin II may occur in combination with extracellular acid production. *S. mutans* and other lactic acid bacteria convert fermentable carbohydrates primarily to lactic acid (for a review see reference 34), and this helps these organisms to maintain their niche. For instance, lactic acid produced by food-grade microorganisms acts as a preservative (26), while lactic acid production by oral streptococci and lactobacilli has been implicated in the dissolution of tooth enamel and thus in the pathogenesis of dental caries (14). Therefore, combinational effects of the bacteriocins and extracellular acid production may suppress growth of competing microbial floras in the respective ecosystems.

In summary, mutacin II is bactericidal and inhibits cell metabolic processes in an unknown, voltage-independent manner, thereby causing cell death without visible lysis.

ACKNOWLEDGMENTS

J.N. and P.W.C. were supported by grants from Unilever Dental Research, United Kingdom, and the National Institutes of Health (NIH grant RO1 DE09082).

We thank Trees Ubbink-Kok for expert technical advice.

REFERENCES

- Au, V., R. G. Carson, B. Harichian, and K. M. Schilling. 1994. Oral hygiene compositions containing antiplaque agents. U.S. patent 5,310,542. Lever Brothers Company.
- Chikindas, M. L. 1995. Unpublished results.
- Chikindas, M. L., M. J. García-Garcera, A. J. M. Driessen, A. M. Ledebor, J. Nissen-Meyer, I. F. Nes, T. Abee, W. N. Konings, and G. Venema. 1993. Pediocin PA-1, a bacteriocin from *Pediococcus acidilactici* PAC1.0, forms hydrophilic pores in the cytoplasmic membrane of target cells. Appl. Environ. Microbiol. **59**:3577–3584.
- Chopin, A., M.-C. Chopin, A. Moillo-Batt, and P. Langella. 1984. Two plasmid-determined restriction and modification systems in *Streptococcus lactis*. Plasmid **11**:260–263.
- Doel, G. 1993. Dentifrice compositions. PCT/international patent application. International publication number WO 93/11738. SmithKline Beecham PLC, United Kingdom.
- Driessen, A. J. M., J. Kodde, S. de Jong, and W. N. Konings. 1987. Neutral amino acid transport by membrane vesicles of *Streptococcus cremoris* is subjected to regulation by internal pH. J. Bacteriol. **169**:2748–2754.
- Driessen, A. J. M., H. W. van den Hooven, W. Kuiper, M. van de Kamp, H.-G. Sahl, R. N. H. Konings, and W. N. Konings. 1995. Mechanistic studies of lantibiotic-induced permeabilization of phospholipid vesicles. Biochemistry **34**:1606–1614.
- Hamada, S., and T. Ooshima. 1975. Production and properties of bacteriocins (mutacins) from *Streptococcus mutans*. Arch. Oral Biol. **20**:641–648.
- Henderson, J. T., A. L. Chopko, and P. D. van Wassenaar. 1992. Purification and primary structure of pediocin PA-1 produced by *Pediococcus acidilactici* PAC1.0. Arch. Biochem. Biophys. **295**:5–12.
- Hirasawa, M., S. Yamaguchi, T. Imanami, I. Matsue, and T. Ikeda. 1984. Effect of mouthrinse containing a bacteriocin from *Streptococcus mutans* on plaque microflora. J. Dent. Res. **63**:298.
- Howell, T. H., J. P. Fiorellini, P. Blackburn, S. J. Projan, J. de la Harpe, and R. C. William. 1993. The effect of a mouthrinse based on nisin, a bacteriocin, on developing plaque and gingivitis in beagle dogs. J. Clin. Periodontol. **20**:335–339.
- Jung, G. 1991. Lantibiotics: a survey, p. 1–34. In G. Jung and H.-G. Sahl (ed.), Nisin and novel lantibiotics. ESCOM Science Publishers B.V., Leiden, The Netherlands.
- Kelstrup, J., and R. J. Gibbons. 1969. Bacteriocins from human and rodent streptococci. Arch. Oral Biol. **14**:251–258.
- Kleinberg, I. 1982. Dental caries, p. 605–624. In W. A. Nolte (ed.), Oral microbiology. The C.V. Mosby Company, St. Louis.
- Konings, W. N., B. Poolman, and A. J. M. Driessen. 1989. Bioenergetics and solute transport in lactococci. Crit. Rev. Microbiol. **16**:419–476.
- Molenaar, D. 1993. Diversity in transport systems. Ph.D. thesis. University of Groningen, Groningen, The Netherlands.
- Molenaar, D., T. Abee, and W. N. Konings. 1991. Continuous measurement of the cytoplasmic pH in *Lactococcus lactis* with a fluorescent pH indicator. Biochim. Biophys. Acta **1115**:75–83.
- Molenaar, D., H. Bolhuis, T. Abee, B. Poolman, and W. Konings. 1992. The efflux of a fluorescent probe is catalyzed by an ATP-driven extrusion system in *Lactococcus lactis*. J. Bacteriol. **174**:3118–3124.
- Moll, G. 1995. Unpublished results.
- Noerlis, Y., and B. Ray. 1994. Factors influencing immunity and resistance of *Pediococcus acidilactici* to the bacteriocin, pediocin AcH. Lett. Appl. Microbiol. **18**:138–143.
- Novák, J., P. W. Caufield, and E. J. Miller. 1994. Isolation and biochemical characterization of a novel lantibiotic mutacin from *Streptococcus mutans*. J. Bacteriol. **176**:4316–4320.
- Novák, J., P. W. Caufield, and E. J. Miller. 1995. Unpublished results.
- Otto, R., B. Klont, B. Ten Brink, and W. Konings. 1984. The phosphate potential, adenylate energy charge and proton motive force in growing cells of *Streptococcus cremoris*. Arch. Microbiol. **139**:338–343.
- Poolman, B., K. J. Hellingwerf, and W. N. Konings. 1987. Regulation of the glutamate-glutamine transport system by intracellular pH in *Streptococcus lactis*. J. Bacteriol. **169**:2272–2276.
- Poolman, B., E. J. Smid, and W. N. Konings. 1987. Kinetic properties of a phosphate-bond-driven glutamate-glutamine transport system in *Streptococcus lactis* and *Streptococcus cremoris*. J. Bacteriol. **169**:1460–1468.
- Ray, B., and W. E. Sandine. 1992. Acetic, propionic, and lactic acids of starter culture bacteria as biopreservatives, p. 103–136. In B. Ray and M. Daeschel (ed.), Food biopreservatives of microbial origin. CRC Press, Boca Raton, Fla.
- Russell, C., and J. R. Tagg. 1981. Role of bacteriocins during plaque formation by *Streptococcus salivarius* and *Streptococcus sanguis* on a tooth in an artificial mouth. J. Appl. Bacteriol. **50**:305–313.
- Sahl, H.-G., R. W. Jack, and G. Bierbaum. 1995. Lantibiotics: biosynthesis and biological activities of peptides with unique post-translational modifications. Eur. J. Biochem. **230**:827–853.
- Sahl, H.-G., M. Kordel, and R. Benz. 1987. Voltage dependent depolarization of bacterial membranes and artificial lipid bilayers by the peptide antibiotic nisin. Arch. Microbiol. **149**:120–124.
- Schuller, F., R. Benz, and H.-G. Sahl. 1989. The peptide antibiotic subtilin acts by formation of voltage-dependent multistate pores in bacterial and artificial membranes. Eur. J. Biochem. **182**:181–186.
- Shinbo, T., N. Kama, K. Kurihara, and Y. Kobatake. 1978. PVC-based electrode susceptible to DDA⁺ as a device to monitor the membrane potential in biological systems. Arch. Biochem. Biophys. **187**:414–422.
- Tagg, J. R. 1991. Studies of “BLIS-full” oral bacteria. N. Z. Dent. J. **87**:14–16.
- Thompson, J. 1976. Characteristics and energy requirements of an α -aminoisobutyric acid transport system in *Streptococcus lactis*. J. Bacteriol. **127**:719–730.
- Venema, G., and J. Kok. 1987. Improving dairy starter cultures. Trends Biotechnol. **5**:144–148.